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EFFECTS OF NUTRIENT ENRICHMENT ON PRIMARY PRODUCTION AND BIOMASS OF SEDIMENT MICROALGAE IN A SUBTROPICAL SEAGRASS BED[1]

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Eutrophication of coastal waters often leads to excessive growth of microalgal epiphytes attached to seagrass leaves; however, the effect of increased nutrient levels on sediment microalgae has not been studied within seagrass communities. A slow-release NPK Osmocote fertilizer was added to sediments within and outside beds of the shoal grass *Halodule wrightii*, in Big Lagoon, Perdido Key, Florida. Gross primary production (GPP) and biomass (HPLC photopigments) of sediment microalgae within and adjacent to fertilized and control *H. wrightii* beds were measured following two 4-week enrichment periods during June and July 2004. There was no effect of position on sediment microalgal GPP or biomass in control and enriched plots. However, nutrient enrichment significantly increased GPP in both June and July. These results suggest that sediment microalgae could fill some of the void in primary production where seagrass beds disappear due to excessive nutrient enrichment. Sedimentary chl *a* (proxy of total microalgal biomass) significantly increased only during the June enrichment period, whereas fucoxanthin (proxy of total diatom biomass) was not increased by nutrient enrichment even though its concentration doubled in the enriched plots in June.

Key index words: **eutrophication; fucoxanthin; gross primary production; *Halodule wrightii*; seagrass; sediment microalgae**

Abbreviations: **DO, dissolved oxygen; GPP, gross primary production; GUIS, Gulf Island National Seashore; NPP, net primary production; *R*, respiration**

Seagrass beds are among the world's most productive coastal ecosystems and support a high diversity of resident autotrophic and heterotrophic species populations. Contributors to primary production within these communities include the seagrasses themselves, assemblages of algal epiphytes attached to seagrass leaves, transient phytoplankton populations in the water column, and sediment microalgae. Within seagrass beds, seagrasses and epiphytes were once thought to be the main contributors to primary production. However, sediment microalgae contribute significantly to total benthic production within these environments. Annual primary production of sediment microalgae has been shown to range from 5 to 892 g $C \cdot m^{-2}$ with a mean of 141 g $C \cdot m^{-2}$ throughout various subtidal and intertidal habitats globally (Webster et al. 2002, Qu et al. 2004).

Pomeroy (1960) was the first to measure primary production rates of sediment microalgae in seagrass beds. At depths >3 m, production rates of sediment microalgae exceeded those of the turtlegrass *Thalassia testudinium*. At depths <2 m, the sediment microalgae, phytoplankton, and *T. testudinium* were of equal importance. Heffernan and Gibson (1983a) and Jensen and Gibson (1986) showed that sediment microalgae within *H. wrightii* (shoal grass), *Syringodium filiforme* (manatee grass), and *T. testudinium* beds in Tampa Bay and the Indian River contributed as much as 85% of the total community primary production. Murray and Wetzel (1987) determined that in the Chesapeake Bay, 14% and 10% of total annual production of *Zostera marina* and *Ruppia maritima* seagrass beds, respectively, were due to sediment microalgae. Daehnick et al. (1992) observed that sediment microalgal production exceeded that of *H. wrightii* in Mississippi Sound seagrass beds, and Pollard and Kogure (1993) made the same observation for *Syringodium isoetifolium* beds in the Fiji Islands. Although primary production of sediment microalgae has been measured a number of times over the last half century, there is no published study that has experimentally determined the role of environmental factors regulating primary production in the sediments of seagrass beds.

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Research focusing on sediment microalgae has consistently reported that small pennate diatoms dominate the assemblage (Daehnick et al. 1992, Moncreiff et al. 1992, Wear et al. 1999, Hansen et al. 2000). These algae influence the ecology of shallow-water aquatic systems, such as seagrass beds, by modifying chemical and physical conditions of surface sediments leading to changes in nutrient cycling (Eyre and Ferguson 2002, Qu et al. 2004). Furthermore, they provide a distinct pathway for secondary utilization of organic matter, which contributes to the heterotrophic assemblages within the seagrass beds (Murray and Wetzel 1987). For this reason, sediment microalgae may be a major food source for lower-level consumers feeding within the seagrass community (Christian and Luczkovich 1999, Moncreiff and Sullivan 2001) as well as in shallow coastal waters lacking seagrass beds (Al-Zaidan et al. 2006, Kang et al. 2006).

Despite the great importance of seagrass beds, these critical communities are rapidly disappearing as a result of increased levels of eutrophication (Sand-Jensen and Borum 1991), specifically anthropogenic elevation of nitrogen (N) concentrations in coastal waters (Short and Burdick 1996, Tomasko et al. 1996, Valiela et al. 1997). According to a NOAA survey, 122 of the 138 U.S. estuaries sampled exhibited symptoms of eutrophication as a result of nutrient enrichment (Bricker et al. 1999). Excess nutrient inputs can alter the biological, chemical, and physical processes that support and maintain aquatic ecosystems, causing a shift in ecosystem structure and function (Nixon 1995). As coastline development continues, N levels will continue to rise (Valiela et al. 1997, McClelland and Valiela 1998) with potential to increase algal biomass within seagrass beds (Wear et al. 1999). *H. wrightii*, the most abundant seagrass species along the coasts of Mississippi, Alabama, and northwest Florida, occurs as medium-sized patchy beds throughout a landscape of microalgal-covered sediments. To date, there is no published study on the effect of nutrient enrichment on sediment microalgae in any seagrass bed. Therefore, we sought to quantify these effects for sediment microalgae assemblages associated with *H. wrightii* beds.

MATERIALS AND METHODS

All work was carried out in monospecific beds of *H. wrightii* along the southern shoreline of Big Lagoon, Perdido Key, Florida (30.325′ N 87.327′ W) in waters <2 m deep. Big Lagoon is a 6.78 km^2 semienclosed lagoon on the western margin of Pensacola Bay within the Gulf Island National Seashore (GUIS) connecting Perdido Bay with Pensacola Bay. Previous studies of seagrass bed production at this site include those of Wear et al. (1999) and Ibarra-Obando et al. (2004). The tidal range within the lagoon is ~0.5 m with low energy regimes in the summer months. Waves result from wind patterns of the system. Summer winds predominantly blow from the southeast toward a barrier protecting the lagoon, causing little current and wave activity during summer months (Ibarra-Obando et al. 2004). Big Lagoon is a brackish water inlet containing monospecific and mixed beds of *H. wrightii* and *T. testudinum*. Recent density measurements of *H. wrightii* and *T. testudinum* ranged from 62.2 to 79.2 g dry weight (dwt) $\cdot m^{-2}$ and 98.3 to 50.2 g dwt $\cdot m^{-2}$ for the two species, respectively, from June to August (Wear et al. 1999).

Nutrient enrichment was simulated in the form of a slow-release Osmocote® fertilizer (IFDC, Muscle Shoals, AL, USA) held in porous tubes anchored horizontally 1–2 cm above the sediment surface. Each enrichment treatment consisted of four porous 40 cm long PVC tubes holding 340 g (0.75 lbs.) of fertilizer wrapped in a nylon stocking. The slow-release fertilizer had the following NPK composition by weight: 15.3% N as $(NH_4)_2SO_4$, 5.0% P as P_2O_5, and 8.3% K as K_2O. The Osmocote fertilizer was enriched with ^{14}N (i.e., ^{15}N-depleted) to serve as a tracer of N (and organic matter) flow within the system. $\delta^{15}N$ values of sediment microalgae, epiphytic algae, and *H. wrightii* leaves indicated rapid uptake of the fertilizer by all three primary producers (M. J. Sullivan, unpublished data).

The fertilizer diffused into the water column overlying the sediments through 30 equidistant 0.6 cm holes drilled through each PVC tube. For each enrichment treatment, four tubes were anchored horizontally in a square formation, and these were placed >2 m away from other treatments. This distance prevented current-induced cross-fertilization contamination (Wear et al. 1999). Such an enrichment protocol has proved to be a highly successful one in seagrass systems (Wear et al. 1999, Heck et al. 2000, Ibarra-Obando et al. 2004, Mutchler et al. 2004), although the horizontal placement of fertilizer tubes as opposed to a vertical placement differed from all four studies cited above. Wear et al. (1999) provided evidence that wave movement and current did not impact fertilizer release or cross-contamination of fertilized and control plots. Therefore, wave and current movements were of trivial impact. Within three replicate *H. wrightii* beds, the fertilizer tubes were placed at the bed center and edge, and at 2 and 6 m from each bed in the ''unvegetated'' sediments. Three additional unfertilized *H. wrightii* beds served as controls for comparison at all four spatial positions. Therefore, there were six experimental beds sampled during this study, with four treatment positions for each bed. The first set of enrichment tubes was placed in *H. wrightii* beds on 25 May 2004. They were replaced by a second set of enrichment tubes with the same enrichment method 4 weeks later on 29 June 2004. Enrichment diffuser tubes have been demonstrated to continuously increase ambient nutrient levels in *H. wrightii* seagrass bed communities for at least 21 d (Mutchler et al. 2004) in Big Lagoon (Wear et al. 1999).

Sediment metabolism was determined ~2 weeks following the placement of the first set of enrichment tubes on 6 June and 7 July 2004, 2 weeks after the second set was anchored in the beds. Dissolved oxygen (DO) readings were taken during in situ incubations inside opaque and transparent 3 L bell jars (Kritter Keepers; Lee's Aquarium, San Marcos, CA, USA) following the methods of Stutes et al. (2006). One transparent (i.e., light) and one opaque (i.e., dark) incubation chamber were pushed 3 cm into the sediments near solar noon. A pair of such bell jars were placed at the four positions in the three control beds and three enriched beds. *H. wrightii* blades were cut immediately above the sediment surface for center and edge positions before bell jar incubations commenced to eliminate seagrass and epiphyte metabolism. DO readings using a WTW OXI 197 dissolved oxygen meter (Global Water Instrument Inc., Gold River, CA, USA) were taken through a drilled hole at the top of each bell jar. At the beginning of each incubation, a DO reading was taken from the light and dark incubation chambers in each bed. The hole was then plugged with a rubber stopper for the duration of the incubation. No less than 3 h later, a second DO reading was taken from both the light and dark bell jars. The initial and final oxygen

readings from the dark chamber provided respiration (R) rates for the sediment community, whereas measurements from the light chamber yielded a direct measurement of net primary production (NPP) of the sediments. NPP and R were calculated using the following equations:

$$\text{NPP} = [(F - I)/T] \times C \times H \times p \quad (1)$$

$$R = [(F - I)/T] \times C \times H \times r \quad (2)$$

where F and I are the DO concentrations ($\text{mg} \cdot \text{L}^{-1}$) at the end and beginning of the incubation period, respectively, T is the incubation time in h, C is the conversion factor of liters to m^3, H is the height of the water column within the incubation chamber (0.15 m), p is the oxygen/carbon conversion factor for community net production of 0.344 mg $\text{C} \cdot \text{mg}^{-1}$ of oxygen based on a photosynthetic quotient of 1 (Strickland and Parsons 1972, Stutes et al. 2006), and r is the oxygen/carbon conversion factor for community respiration of 0.375 mg $\text{C} \cdot \text{mg}^{-1}$ oxygen based on a respiratory quotient of 1 (Strickland and Parsons 1972, Stutes et al. 2006). GPP within the sediments was calculated as NPP − R. All three measurements of community metabolism were expressed as mg $\text{C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. NPP and R of the phytoplankton trapped within the light and dark chambers were corrected by incubating four light and four dark BOD bottles, respectively, above the sediment surface at the midpoint of the chambers for 3 h periods. BOD measurements in four light and four dark bottles were collected during both June and July measurements of coinciding GPP of sediment microalgae.

Sediment microalgal photopigments were measured 3 weeks following placement of the first set of enrichment tubes on 21 June and 17 July 2004, 3 weeks after the second enrichment. Although it would have been beneficial to sample both primary production and photopigments of sediment microalgae during the same collecting week, collections were made with 2 weeks of each other. However, previous studies (Wear et al. 1999, Mutchler et al. 2004) provided evidence that enrichment diffuser tubes will continuously release fertilizer into the water column and sediments for at least 21 d. Temperature from Big Lagoon varied little between collection dates and was consistently between 27°C and 28°C for both June and July sampling. Sediment cores (2.5 cm in diameter) were extracted from each bed at all locations—center, edge, and 2 and 6 m from the bed edge. For each core, the top 3 cm of sediment was sliced onto a Whatman GF/F glass-fiber filter (pore size = 0.7 μm; Maidstone, Kent, UK) and immediately hand-filtered with a portable vacuum pump in the field. This dehydration reduces the variability in pigment concentration caused by differing water contents of sampled sediments. HPLC has proved to be highly effective in separating and quantifying photosynthetic pigments that are diagnostic of resident algal groups (Jeffrey et al. 1997), and a modification of this methodology was used herein (Zimba et al. 2002). Such diagnostic pigments include chl a for total algal biomass, fucoxanthin for diatoms, lutein and chl b for green algae, and zeaxanthin for cyanobacteria (Wear et al. 1999). HPLC values for the cores were extrapolated to an aerial basis (e.g., $\text{mg} \cdot \text{m}^{-2}$). All seagrass leaf fragments encountered while coring were discarded to exclude seagrass pigments from HPLC analysis.

The data consisted of GPP rates ($\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) and two sets of photosynthetic pigment concentrations, chl a and fucoxanthin ($\text{mg} \cdot \text{m}^{-2}$), from the center, edge, and 2 m and 6 m from the edge of enriched and control beds. Using SyStat software (Chicago, IL, USA), a repeated measure three-way analysis of variance (ANOVA; treatment × position × date) was used to analyze these data. Testing for statistical significance of component tests is routinely carried out in a biased fashion that liberally judges the significance of the test (Rice 1988). Therefore, to ensure confidence in the ANOVA P-values, the sequential Bonferoni correction was applied to P-values across the GPP, chl a and fucoxanthin responses to treatment, and position and effect of date where these variables were significant.

RESULTS

A distinct carpet of sediment microalgae was apparent in the sandy sediments beneath the canopy of *H. wrightii* and continued into the seemingly unvegetated sediments. A golden-brown color was readily apparent on the sediment surface throughout the study site. Microscopic observation of sediments showed that small pennate diatoms dominated the sediment microalgal assemblages. Occasionally, cyanobacteria were scattered throughout the diatom assemblage. SEM revealed a taxonomically diverse flora of biraphid, monoraphid, and araphid diatoms. Qualitative SEM observations indicate that the most abundant diatom genera in terms of number of individuals were *Amphora*, *Diploneis*, *Fallacia*, *Navicula*, *Nitzschia*, *Cocconeis*, *Opephora*, and *Mastogloia*.

GPP of sediment microalgae exposed to the Osmocote fertilizer ranged from 23 to 136 mg $\text{C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. In unfertilized control beds, sediment microalgae GPP ranged from 15 to 155 mg $\text{C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. Three-way ANOVA showed significant effects of date and treatment, but no interaction term was significant (Table 1). One-way ANOVA for June and July revealed a significant effect of nutrient enrichment ($P = 0.002$ and 0.005, respectively; Table 2). On each date, sediment microalgae GPP was significantly higher in fertilized plots (Fig. 1). GPP doubled due to enrichment during the June sampling. Sediment microalgal GPP was statistically equivalent for plots within (center and edge) and outside (2 m and 6 m from the edge) *H. wrightii* beds, as the effect of position was not significant (Tables 1 and 2).

TABLE 1. Three-way analysis of variance (ANOVA) results for gross primary production (GPP) of sediment microalgae at four spatial positions in enriched and control *Halodule wrightii* beds on 6 June and 7 July 2004 from Big Lagoon, Perdido Key, Florida.

	SS	df	MS	F	P-value
Date	14,480.594	1	14,480.594	19.072	**<0.001**
Treatment	18,785.613	1	18,785.613	24.741	**<0.001**
Position	2,089.798	3	696.599	0.917	0.443
Date × treatment	371.442	1	371.442	0.489	0.489
Date × position	4,239.521	3	1,413.174	1.861	0.156
Treatment × position	346.821	3	115.607	0.152	0.927
Date × position × treatment	1,456.068	3	485.356	0.639	0.595

Numbers in bold indicate statistically significant P-values.

TABLE 2. One-way analysis of variance (ANOVA) results for gross primary production (GPP) of sediment microalgae at four spatial positions in enriched and control *Halodule wrightii* beds on 6 June and within 7 July 2004 from Big Lagoon, Perdido Key, Florida.

	SS	df	MS	*F*	*P*-value
June					
Treatment	12,220.073	1	12,220.073	14.022	**0.002**
Position	1,997.332	3	665.777	0.482	0.698
Treatment × position	1,450.932	3	483.644	0.555	0.652
July					
Treatment	6,936.982	1	6,936.982	10.721	**0.005**
Position	4,331.987	3	1,443.996	2.232	0.124
Treatment × position	351.957	3	117.319	0.181	0.908

Numbers in bold indicate statistically significant *P*-values.

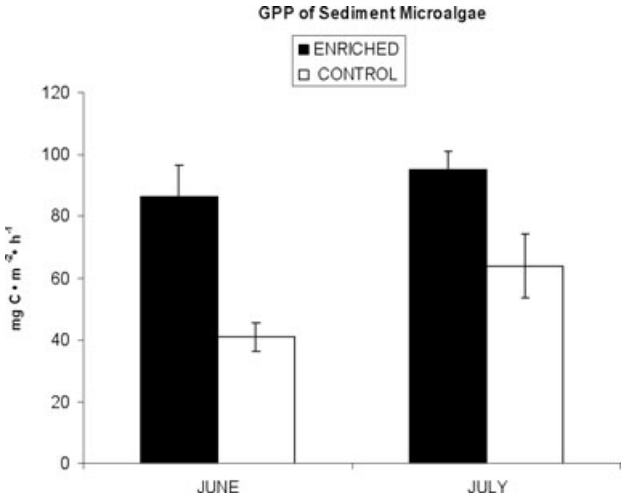

FIG. 1. Mean gross primary production (GPP; $n = 12$) for sediment microalgae within and outside enriched and control *Halodule wrightii* beds in Big Lagoon, Perdido Bay, Florida, on 6 June and 7 July 2004. Standard errors accompany each mean value.

Chl *a* concentrations, a proxy for total sediment microalgae biomass, ranged from 13 to 73 mg · m^{-2} in enriched sediments and from 8 to 53 mg · m^{-2} in control sediments. Three-way ANOVA showed that date and date × treatment were significant ($P = 0.011$ and 0.016, respectively, see Table 3). One-way ANOVA revealed that there was a significant treatment effect only in June ($P = 0.004$, see Table 4). Nutrient enrichment in June significantly increased chl *a* concentrations in the sediment but had no effect on the standing crop of sediment microalgae in July (Fig. 2). Position (inside vs. outside the bed) was not a significant variable (Tables 3 and 4).

Fucoxanthin concentrations, a proxy for diatom biomass, ranged from 1 to 13 mg · m^{-2} in enriched plots and 0.8–14 mg · m^{-2} in control plots. Three-way ANOVA revealed that only date was a significant explanatory variable accounting for fucoxanthin variation ($P < 0.001$, see Table 5). Although there was no effect of enrichment, fucoxanthin concentrations doubled in the enriched plots in June (Fig. 3). As was the case for GPP and chl *a*, position effects were not significant (Table 5).

TABLE 3. Three-way analysis of variance (ANOVA) results for chl *a* of sediment microalgae at four spatial positions in enriched and control *Halodule wrightii* beds on 21 June and 17 July 2004 from Big Lagoon, Perdido Key, Florida.

	SS	df	MS	*F*	*P*-value
Date	183.985	1	183.985	1.342	**0.011**
Treatment	989.674	1	989.674	1.342	0.255
Position	985.993	3	328.664	2.397	0.086
Date × treatment	882.205	1	882.205	6.443	**0.016**
Date × position	444.571	3	148.190	1.018	0.371
Treatment × position	315.718	3	105.239	0.767	0.521
Date × treatment × position	82.254	3	27.418	0.200	0.896

Numbers in bold indicate statistically significant *P*-values.

TABLE 4. One-way analysis of variance (ANOVA) results for chl *a* of sediment microalgae at four spatial positions in enriched and control *Halodule wrightii* beds on 21 June and 17 July 2004 from Big Lagoon, Perdido Key, Florida.

	SS	df	MS	*F*	*P*-value
June					
Treatment	1,870.335	1	1,870.335	11.142	**0.004**
Position	625.328	3	208.443	1.242	0.327
Treatment × position	244.848	3	81.616	0.486	0.697
July					
Treatment	1.544	1	1.544	0.015	0.906
Position	805.236	3	268.412	2.522	0.095
Treatment × position	153.125	3	51.042	0.480	0.701

Numbers in bold indicate statistically significant *P*-values.

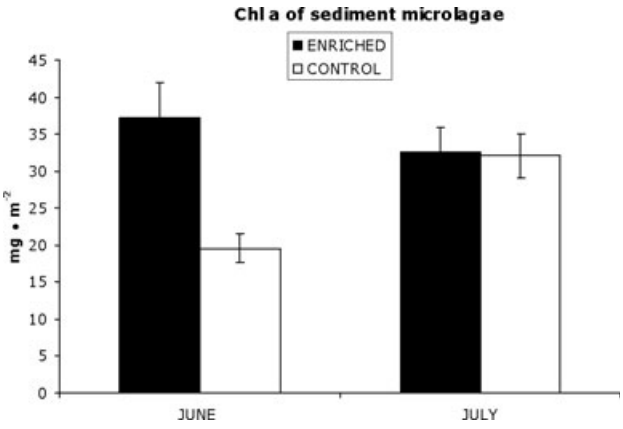

FIG. 2. Mean chl *a* ($n = 12$) for sediment microalgae within and outside enriched and control *Halodule wrightii* beds in Big Lagoon, Perdido Bay, Florida, on 21 June and 17 July 2004. Standard errors accompany each mean value.

DISCUSSION

The sediment microalgae assemblages beneath the leaves of *H. wrightii* and outside the seagrass

TABLE 5. Three-way analysis of variance (ANOVA) results for fucoxanthin of sediment microalgae at four spatial positions in enriched and control *Halodule wrightii* beds on 21 June and 17 July 2004 from Big Lagoon, Perdido Key, Florida.

	SS	df	MS	*F*	*P*-value
Date	213.001	1	213.001	34.712	**<0.001**
Treatment	2.985	1	2.985	0.482	0.493
Position	23.677	3	7.892	1.286	0.296
Date × treatment	15.633	1	15.633	2.584	0.120
Date × position	15.938	3	5.313	0.866	0.469
Treatment × position	11.238	3	3.746	0.610	0.613
Date × position × treatment	1.078	3	0.359	0.058	0.981

Numbers in bold indicate statistically significant *P*-values.

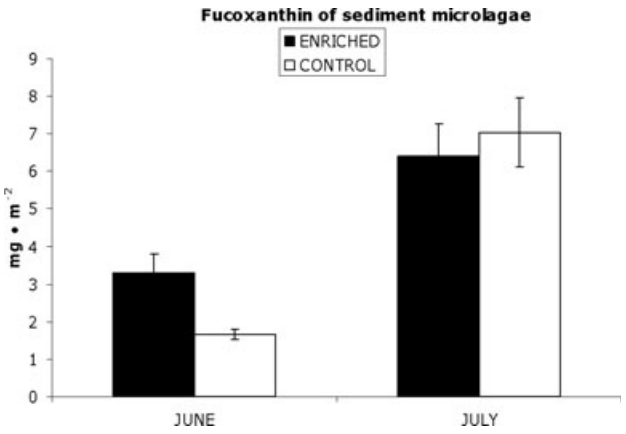

FIG. 3. Mean fucoxanthin ($n = 12$) for sediment microalgae within and outside enriched and control *Halodule wrightii* beds in Big Lagoon, Perdido Bay, Florida, on 21 June and 17 July 2004. Standard errors accompany each mean.

beds in seemingly unvegetated sediments were dominated by small pennate diatoms. This finding is consistent with previous studies in sandy sediments of subtidal marine habitats (Sundbäck 1984, Delgado 1989, Daehnick et al. 1992). The carpet of sediment microalgae was consistent throughout the entire study site; there were no readily observable differences between species inhabiting sediments within and outside *H. wrightii* beds based on qualitative SEM observations.

Previous evidence of effects of nutrient enrichment on primary production and biomass of seagrasses and their epiphytes from Big Lagoon are comparable to the trends of the current study. Wear et al. (1999) observed a significant increase in primary production of three seagrass species from Big Lagoon, including *H. wrightii* due to increased nutrient enrichment inputs from the same Osmocote® diffuser tube technique used here. Primary production output of seagrass epiphytes in Big Lagoon was also significantly increased due to nutrient enrichment. However, there was no significant change in seagrass biomass due to nutrient enrichment. Wear et al. (1999) also determined epiphytic biomass to be significantly increased due to nutrient enrichment. Epiphytic biomass was measured via HPLC pigment analysis of chl *a*. Due to the recent measurements of effects of nutrient enrichment on both seagrass and epiphytic biomass and primary production throughout Big Lagoon, Florida, this study focused on these parameters.

It is of interest to compare production rates of control sediment microalgae to those measured in other seagrass systems because of the paucity of such measurements compared with their epiphytic counterparts. Since this study only sampled during June and July, it is best to make these comparisons based on hourly rates. Pomeroy (1960) and Heffernan and Gibson (1983a,b) reported ranges of 8–47 and 3–34 mg $C \cdot m^{-2} \cdot h^{-1}$, respectively, while Jensen and Gibson (1986) found a maximum value of 45 mg $C \cdot m^{-2} \cdot h^{-1}$ in Florida seagrass beds. Daehnick et al. (1992) measured rates of 9 to 276 mg $C \cdot m^{-2} \cdot h^{-1}$ over an annual cycle. Sediment microalgae GPP in this study ranged from 15 to 155 mg $C \cdot m^{-2} \cdot h^{-1}$ in control plots, which is similar to those values given in previous studies. The mean production rate for the sediment microalgae from this study was 52 mg $C \cdot m^{-2} \cdot h^{-1}$. The grand mean for sediment microalgal production reported by Heffernan and Gibson (1983a,b), Murray and Wetzel (1987), and Daehnick et al. (1992) was 17, 37, and 80 mg $C \cdot m^{-2} \cdot h^{-1}$, respectively. Again, the short-term data generated by this study closely match those of previous ones.

Nutrient enrichment stimulates GPP of different assemblages of benthic microalgae (Menge 1992, Pinckney et al. 1995), but this phenomenon has not been tested for sediment microalgae in seagrass communities. In this study, nutrient enrichment significantly increased GPP of sediment microalgae in both June and July, with a doubling of GPP in enriched sediments during June. One might have expected the difference between GPP in control and enriched beds would be greater in July than in June because of the constant exposure of the sediment microalgae to nutrient enrichment throughout the field experiment. Results for sediment photopigments may provide part of the explanation (see below). It is possible that the summer influx of N from offshore runoff as measured from the Pensacola Waste Water Treatment Plant at Pensacola Beach, <5 km from the study site, from June (4.7 mg $\cdot L^{-1}$) to July (6.6 mg $\cdot L^{-1}$) 2004 increased nutrient loading into Big Lagoon, thus decreasing the effects of experimental enrichment. Nutrient enrichment of Big Lagoon sediments strongly influenced GPP of sediment microalgae, corroborating reports that N enrichments cause production changes in microalgal communities in habitats ranging from coral reefs (Miller et al. 1999) to marine microbial mats (Pinckney et al. 1995) as well as in macroalgal communities from diverse habitats (Valiela et al. 1997).

HPLC provides useful information concerning microalgal physiological status and community composition. The responses of sediment chl *a* and fucoxanthin concentrations paralleled one another in this study. The doubling effect seen in June for chl *a* in enriched plots also characterized fucoxanthin concentrations in enriched plots in June. Control plots in July exhibited increases in chl *a* and fucoxanthin concentrations to levels equaling or exceeding those found in enriched June plots. These parallel results for chl *a* and the carotenoid pigment fucoxanthin indicate that chl *a* and fucoxanthin concentrations provide reliable proxies for sediment microalgae biomass where diatoms dominate the assemblage.

Chl *a* in Big Lagoon ranged from 8 to 73 mg · m^{-2}, with an overall mean of 30 mg · m^{-2}. The control plots possessed chl *a* concentrations ranging from 8 to 53 mg · m^{-2}, with a mean of 22 mg · m^{-2}. Previous studies have observed chl *a* values comparable to the ones reported here, whereas others have measured much greater values. Daehnick et al. (1992) extracted chl *a* from subtidal sandy sediments within *H. wrightii* beds in Mississippi Sound and reported chl *a* concentrations ranging from 14 to 125 mg · m^{-2} with a mean of 44 mg · m^{-2}. These concentrations are for the most part lower than those measured in sediments shaded by a vascular plant canopy in salt marshes (Sullivan and Currin 2000, Wozniak et al. 2006). Higher values for subtidal sandflats devoid of macrophytes have also been measured: 23–258 mg · m^{-2} (Sundbäck 1984) and 11–340 mg · m^{-2} (Cahoon and Safi 2002). However, subtidal sediments of the Loch Ewe in Scotland (Steele and Baird 1986) and Laholm Bay in Sweden (Sundbäck and Jönsson 1988) yielded chl *a* concentrations well below 100 mg · m^{-2}. This variability probably reflects a suite of environmental factors interacting with sandy substrata in these different areas, such as nutrient availability, grazing, physical disturbance, and irradiance patterns (Cahoon and Safi 2002).

Fucoxanthin concentrations in this study were rather low and ranged from 0.8 to 14 mg · m^{-2}, with a mean of 4.6 mg · m^{-2}. In control plots, the mean fucoxanthin concentration was 4.3 mg · m^{-2}. Armitage and Fong (2004) reported a mean fucoxanthin concentration of 58 mg · m^{-2} within enclosures on intertidal sandy sediments, which is an order of magnitude higher than reported here. Seasonal sampling and the lack of grazers within the control enclosures used by Armitage and Fong (2004) may explain the difference. This difference also suggests the possibility of a top-down effect within Big Lagoon (i.e., mesograzers, such as shrimp, as well as deposit and suspension feeders both within and outside seagrass beds are consuming diatom biomass).

Nutrient enrichment significantly increased concentrations of chl *a* in June but not July. Chl *a* biomass doubled in enriched plots in June. Although we detected no significant effect of treatment or treatment interactions on sedimentary fucoxanthin, the concentration of this pigment also doubled in the enriched plots in June (Fig. 3). This finding may explain why GPP of the sediment microalgae more than doubled due to enrichment in June, whereas the increase in July, although significant, was less pronounced. A higher standing crop of sediment microalgae should have a higher potential GPP. Although it has generally been assumed that algal biomass should increase with N enrichment, previous field manipulations of sediment microalgae have produced different outcomes. Estrada et al. (1974) sampled chl *a* concentrations beneath *Spartina alterniflora* in a Massachusetts salt marsh during late summer (July–September) and found that organic N amendments did not increase benthic microalgae biomass. However, inorganic N enrichment of the sediment between the same macrophyte species in a Delaware salt marsh in spring and early summer did increase the standing crop of sediment microalgae (Sullivan 1976), similar to results of this study. The lack of a response of chl *a* and fucoxanthin concentrations to nutrient enrichments during July in Big Lagoon could be due to an increased influx of nutrients into Big Lagoon as suggested by the rise in chl *a* and fucoxanthin in control plots (Figs. 2 and 3). This peak coincided with an increased wastewater N discharge during July in this region. Underwood et al. (1998) reported no consistent pattern of benthic microalgal responses to nutrient enrichment, similar to what this study has reported. Sullivan (1981) found that N enrichment in a Mississippi salt marsh had virtually no effect on sediment microalgal biomass, and Christian et al. (1978) demonstrated that the sediment microbial assemblage was resistant to N additions. However, Armitage and Fong (2004) observed that nutrient enrichment significantly elevated levels of sedimentary fucoxanthin and chl *a*, as was the result in this study for the June sampling.

An unexpected result was that positional differences of enrichment inside the bed, on the edge of the bed, and in the two positions outside the bed in open sediments did not affect sediment microalgal GPP or biomass. Despite potential differences in environmental and physiological factors, such as light availability and seagrass metabolism, respectively, GPP and photopigment concentrations were statistically equivalent within and outside *H. wrightii* beds (position *P*-values, Tables 1–5). A multiple regression analysis by Daehnick et al. (1992) showed that light was selected as an explanatory variable for sediment microalgal production in *H. wrightii* beds; however, it only explained 6% of the variance in production. Furthermore, sediment chl *a* concentrations were not selected by the model. Lindeboom and Sandee (1989) measured relatively high production rates for sediment microalgae adjacent to Indonesian seagrass beds and concluded their overall contribution within

the dense beds was small. It would be of interest to test their assumption and determine when canopy shading reduces sediment microalgal production to levels below those measured outside the beds. The reduction of light by the leaf canopy of *H. wrightii* in Big Lagoon appeared to have no effect on sediment microalgal GPP, suggesting that this algal assemblage may be shade adapted.

This study provides evidence that sediment microalgae within and adjacent to subtropical seagrass beds may be stimulated by nutrient enrichment. Excessive nutrient loading has been shown in some cases to cause seagrass beds to disappear and be replaced by opportunistic macrolagae (Cardoso et al. 2004). Blooms of such algae would likely be short lived or at least seasonal, but sediment microalgae would persist year-round. In some systems, sediment microalgal production may equal that of macroalgae (Glud et al. 2002). Along with macroalgae, sediment microalgae may be capable of filling some of the void of lost primary production resulting from loss of seagrasses and their epiphytic algae, although the loss of those production components, especially the seagrasses themselves, would be detrimental to the production output of the system and could not be compensated by the sediment microalgae alone.

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